

Identification of *Taxus cuspidata* Sieb. et Zucc endophytic fungi—new recorded-genus-species of China and the metabolite

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Abstract: A total of 94 isolates of endophytic fungi were isolated from the bark of 200-yr.-old *Taxus cuspidata* Sieb. et Zucc. in the primeval forest of the Changbai Mountain Natural Reserve, and 19 species of endophytic fungi were identified. Among the taxa 2 new species and 1 new varied species as well as the 6 known species of China had been described in a previous article published on Journal of Forestry Research, Vol. 14 (4): 290-294 (2003). This paper made a detail description on the morphological characteristics of the 9 new recorded-genus-species and 1 new record species with illustrations, including *Staphylotrichum coccosporum* Meyer et Nicot, *Botryodema lateritium* Papendooft & Upadhyay, *Oedocephalum glomerulosum* (Rul-liard) Sacc., *Trichosporonoides oedocephalis* Haskins & Spencer, *Beniowskia sphaeroidea* (Kal Chbrenner et Caeke) Mason, *Thermomyces verrucosus* Pugh, Blakeman & Morgan-jones, *Gilmaniella humicola* Brnon, *Arthrinium sphaerosperma* (Per.:Fr.) Ellis, *Ambrosiella brunnea* (Verrall) Batra, and *Cytosporina notha* Diedz (1 new record species). And the habitats, hosts, distributions and fruiting periods were recorded for the new recorded-genus-species. The determination result of thin layer chromatography (TLC) showed that the liquid fermentation metabolites of the strains could not react positively with taxoids developer - the vanillic aldehyde and dilute bismuth potassium iodide.

Keywords: *Taxus*; Endophytic fungi; Identification; New recorded-genus-species of China

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Materials and methods

Sources of *Taxus* specimens

Isolation specimens were collected from *Taxus cuspidata* Sieb. et Zucc. in the primeval forest of the Changbai Mountain Natural Reserve. From 1996 to 2000, the bark specimens of different tissues of *T. cuspidata* Sieb. et Zucc. was collected, numbered and reserved in paper bags.

Media

The following media were prepared and used in the experiment.

Solid-state media

(1) PDA: 200-g potato, 20-g glucose, 20-g agar, and 1000-mL distilled water, with natural pH. Sterilization was carried out at 121 °C for 20 min.

(2) Ciaolk's Agar: 30-g sucrose, 3-g NaNO₃, 0.5-g KCl, 0.01-g Fe₂(SO₄)₃, 0.5-g MgSO₄, 1-g KH₂PO₄, 20-g agar and 1000-mL distilled water, with natural pH, sterilized at 121°C for 20 min.

(3) Corn meal-Sucrose Agar: 40-g Corn meal, 10-g sucrose, 20-g agar and 1000-mL distilled water, with natural pH, sterilized at 121°C for 20 min.

Liquid production media

(1) Seed medium was prepared with glucose (20 g), peanut (10 g) powder (10 g), MgSO₄ (3 g), KH₂PO₄ (3 g), NH₄Cl (3 g) and distilled water of 1000 mL, with natural pH, and sterilized at 121 °C for 20 min.

(2) Production medium: glucose (10 g), peanut powder (10 g), soybean powder (10 g), starch (10 g), MgSO₄ (3 g), KH₂PO₄ (3 g), NH₄Cl (3 g) and distilled water of 1000 mL, with natural pH, were used and sterilized at 121 °C for 20 min.

Methods

Isolation of endophytic fungi

(1) Isolation: The specimens of *T. cuspidata* were dipped in 75% ethanol for 1 min, and flamed on burning alcohol for 15 s. The inner section excised surface layer was put on the PDA solid media with antibiotic, such as, 100 µg/mL penicillin or 40 µg/mL streptomycin, or on the other solid media in Petri dishes, and incubated at 25 °C in the dark for some days. The tip of fungal hypha growing out from the inner bark was incubated at 25 °C and cultured onto the fresh PDA in culture tube. After several times of repeated subculture, the isolates were obtained.

(2) Identification: The ontogeny characteristics of hypha, conidiogenous structure, conidia during the culture were observed and described through direct-pick processing and slide-insert culture (Xiang 1991). The genus and the species of the endophytic fungi were discerned, based on the literature retrieving (Barnett *et al.* 1972; Ainsworth *et al.*

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1973; Von Arx. 1974).

Screening identification of fermentation metabolite

The isolates were screened through TLC described by as follows. The target strains were screened by following methods as: i) the thin layer slates were directly put under UV light with 253.7 nm and 376.5 nm; ii) NO.1 developer vanillic aldehyde were sprayed on the thin layer slates then the thin layer slates were heated at 100 °C for 8 mins. iii) NO.2 developer dilute potassium bismuth iodide were sprayed on the thin layer slates (Xiang 2002, 2003).

Results

A total of 94 isolates were isolated from *Taxus cuspidata* Sieb. et Zucc. and 19 species were identified, composed of 2 new species, 1 new varied species, 9 novel recorded-genus in China and 10 novel recorded-species in China.

Description of new recorded-genus-species of China

(1) *Staphylotrichum* J.Meyer & J.Nicot, Bull. Soc. Mycol. Fr. 72:318-323, 1957; Maciejowska & Williams, Mycologia, 55: 221, 1963, new recorded-genus of China

Characteristics: Colonies were flat and radical extension. The mycelia were wooly, sparse and initially hyaline, and then turning gradually gray white in color. The substratum was pale-yellow in color. Conidiophore was irregular branched, hyaline and septate. The conidium was terminal solitary aleuriospores, unicellular, hyaline, globose and smooth thick-walled.

Type species: *Saphylotrichum coccosporum* Meyer et Nicot

Staphylotrichum coccosporum Meyer et Nicot, new recorded-species of China (Nicot et Meyer, 1957; Barron 1968) in Fig. 1-A.

Characteristics: Colonies were short wooly-like to wooly-like, and had the color from hyaline to grayish white. The substratum was pale-yellow in color. The mycelia were branched, from hyaline to lightly-yellow in color, which were 3-6 µm broad. The conidiophores were straight, smooth, septate in shape, about 1 mm long, and its basal part was broader, 5-8 µm in diameter. The conidiogenous cell was lithly-filiform, short, 3-3.5 µm in diameter. The conidium had characters such as solitary teminal aleurio-spores, unicellular, hyaline, solitary, globose to subglobose, 8-12 µm long, thick-walled and smooth.

Specimen: The bark HSSPD 1012 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(2) *Botryodema lateritium* Papend.& Upadhyay, Trans. Br. Mycol. Sos. 52: 257-265, 1969, new recorded-genus of China

Characteristics: Colonies were loose, round, flat and short wooly. The hyphae were sparse from hyaline to reddish brown in color, with septate and thick-walled in substratum with dark reddish brown. The conidiospores with lateral or apical sporogenous cell had various shape such as short, subglobose, obpyriform, clavate or subclinder. Aleurio-spore was unicellular and apically or laterally born on the short sterigma, from broad-ellipse to subglobose in shape, of which subhyaline was smooth with convex scar.

Type species: *Botryodema lateritium* Papend.& Upadh.

Botryodema lateritium Papendoorf & Upadhyay, new recorded-species of China (Papendorf *et al.* 1969) in Fig. 1-B.

Characteristics: Colonies were cultured on malt agar or PDA. At first their aerial mycelia were colourful or colourless, then became very light fumeus, and the central region of the colonies slightly rose and formed a powdery spongiosum granulos mat with a rosy lateritius colour, often with concentric wheel. Hyphae were branched with the characters of thin-walled, smooth, and septate, hyaline and in diameter of 1.5-4 µm. In conidiogenous cell region of young colony, mycelia were branched and formed dense clusters. The near axis regions of main fascicular hypha with length of 30-40 µm had a number of short conidiophores and numerous lateral sporogenous cells that were single, thick-walled, hyaline, basally septate and usually inflated, from subglobose to obpyriform in shape and 3-4 µm in diameter. These short conidiophores and numerous lateral sporogenous cells produced 1-4 aleuriospores, which were oblong-elliptical, ovoid or subglobose, smooth, thick-walled, hyaline or subhyaline and (4-8)×(4-6) µm in size, and there are convex scar at the base.

Specimen of study: The bark HSSPD 1002 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(3) *Oedocephalum* Preuas, Linnaea 24:131, 1851, new recorded-genus of China

Characteristics: Colonies were extensive in shape with white or little black patches in color. And substratum was reddish brown. Mycelium was hyaline, the terminal of conidiophore was unbranched and swell into globose in shape. The terminal of conidiophore grew with the dried conidial heads together, and appeared some denticles after the shedding of conidia that were botryblastospores, hyaline, unicellular, globose to oval globose. The telemorph was Descomycetes—Peziza—Pyronerma and Hymenomycetes—Heterobasida—Fomes.

Type species: *Oedocephalum glomerulosum* (Bull.:Fr.) Sacc.

Oedocephalum glomerulosum (Bull.:Fr.) Sacc., new recorded-species of China (Saccardo 1882-1931; von Arx. 1974; Barron 1968) in Fig. 1-C.

Characteristics: Colonies were radical extension, with

compacted small particle-like spores masses and white in color. Substratum was reddish brown in color. Hyphae were hyaline, branched and septate. The conidiophores were filiform, 50-120 μm in length, and the terminal of the conidiophore was swell into globose conidiogenous cell—ampulla of 9-12 μm in length. The conidiogenous cell was born with conidial heads together, appeared denticle when the conidia shed. Conidium was botryoblastospores, lightly orange-red in color, unicellular, from globose to oval in shape, 3-4 μm long.

Specimen of study: The bark HSSPD 1016 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(4) *Trichosporonoides* Hskins & Spencer, Can. J. Bot. 45:515-520, 1967; Hoog, Stud. Mycol. 15:20, 1979, new recorded-genus of China

Characteristics: Colonies were sparse, wooly, rat gray in color, with thick patch-like conidia masses. The colonies formed by hyphae had a characteristic with a thin layer appressed on the substratum, which can break into hyaline pseudohyphae. Conidiophore whose terminal part was always swollen globose, then grew with botryospores on sterigmata, or broke into anthrospores and yeast-like blastospores too.

Type species: *Trichosporonoides oedocephalis* Hskins & Spencer

Trichosporonoides oedocephalis Hskins & Spencer, new recorded-species of China (Haskins *et al.* 1967) in Fig. 1-D.

Characteristics: Sprout cell was hyaline, from oval to ellipse in shape, (3.5-5) \times (5-10) μm in size. Mycelium, 3-3.5 μm in diameter, can form pseudomycelium. The anthrospores of (3-3.5) \times (4-10) μm were smooth, and solitary blastospore was 4-5 μm long. The conidiophore was 10-250 μm long, and the terminal of conidiophore was swell into brown globose, and 8-12 μm in length. The little sterigma can bear fascicle botryospores, or form chain-like blastospores on the conidiophore, or botryospores.

Specimen of study: The bark HSSPD 1023 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(5) *Beniowskia* Raciborski, Paras Algen Pilze Javas, 2:37, 1900, new recorded-genus of China

Characteristics: Colonies were sparse, wooly, flat, initially pale-cassia, gradually fuscous, composing of hair-like, hyaline mycelia. The substratum was raw umber. Conidiophores were curved (flexuous), unbranched and hyphal-like. Conidiogenous cells were hyohoid. Conidium was blastospore, lateral, globose and hyaline

Type species: *Beniowskia sphaerodes* (Kal Chbrenner &

Cooke) Mason=B. graminis Raciborsky.

Beniowskia sphaerodes (Kal Chbrenner & Cooke) Mason=B. graminis Raciborsky, new recorded-species of China (Mason 1927) in Fig. 1-E.

Characteristics: Colonies on PDA, initially pale-cassia, subsequently turning to dark cassia to dark cassia-orange, made the substratum fuscous. Hair-like mycelia were soft-walled, smooth, septate, and 3-10 μm long. Conidium was blastospore, lateral, globose, hyaline and smooth, 16-20 μm long.

Specimen of study: The bark HSSPD 1004 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(6) *Thermomyces* Tsiklinsky, Ann. Inst. Pasteur, 15: 500-505, 1899; Pugh *et al.*, Trans. Brit. Mycol. Soc. 47(1): 115-121, 1960, new recorded-genus of China

Characteristics: Compacted colonies, initially buff-yellow to pale-red, subsequently reddish brown, were always wooly with conspicuous water-drops, thick black spore masses and substratum in the reverse was dark reddish brown in color. The mycelium was thick, septate, hyaline to subhyaline, lateral or apical. Conidiophore was unbranched or occasionally branched, hyaline or subhyaline, and the terminal of conidiophore gave rise to solitary aleuriospores, which were globose or subglobose, brown in color, unicellular, thick-walled and rough.

Type species: *Thermomyces lanuginosus* Tsiklinsky

Thermomyces verrucosus Pugh, Blakeman & Morgan-jones, new recorded-species of China (Tsiklinsky 1899; Pugh, Blakeman et Morgan-jones 1964) in Fig. 1-F.

Characteristics: Colonies on PDA at 25 °C were profuse short wooly, light red to dark reddish brown in color, with conspicuous drops of water on the surface and thick black particle-like spores masses. Hyphae were thick, septate, hyaline or subhyaline, and had apically or laterally unbranched conidiophores of 7-10 μm in length, or occasionally branched conidiophores of 20-30 μm in length. Terminal cell of conidiophore slightly swelled to annellophore, bearing single aleuriospores that were globose, unicellular, dematiaceous with conspicuous water-drops, and the outer wall of aleuriospore broke to floccular 14.5 (10-17) μm long when it matured.

Specimen of study: The bark HSSPD 1025 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(7) *Gilmaniella* Brnon, Mycologia, 56:514-516. 1964, new recorded-genus of China

Characteristics: Colonies were sparse, long wooly, initially white to gray, turning rapidly brown, finally brown with ages. Vegetative hyphae were hyaline to brown with

marked septa. Conidiospores were hyaline, short solitary or unbranched, often inflated. Conidia had solitary apical, single or clusters of aleuriospores, dark-brown, with germ pore.

Type species: *Gilmaniella humicola* Barron

Gilmaniella humicola Barron, new recorded-species of China (Barron 1964, 1968) in Fig. 1-G.

Characteristics: Colonies grown on PDA at 25 °C can reach 5-6 cm in diameter, at first whitish to pale-gray, quickly became pale-brown, initially white to gray, rapidly turning to brown and finally fuscous with ages. Numerous water droplets were common on the aerial hyphae. Vegetative hyphae were hyaline to brown, 1.5-5 µm long, marked septa with ages. Conidiophores were simple, filiform or clavate, sometimes branched, sporogenous cell and stalk cell frequently inflated, with 5-25 µm long and 1.5-3 µm broad. Conidia presented apical and solitary aleuriospores, dark-brown, smooth, unicellular of 12-15 µm in length, with marked apical germ pore.

Specimen of study: The bark HSSPD 1022 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(8) *Arthrinium* Kunze., Mycol.Hefte 1:9, 1817; Syno: *Papularia* Fries, 1825; Ellis, M.B. Dematiaceous hyphomycetes in Mycol. Papers, CMI 103: 1-46, new recorded-genus of China

Characteristics: Colonies were flat, woolly, sparse, initially white, gradually turning black, with compacted black mycelium masses and substratum was black. Most of conidiophores were unbranched, arthric, septate, hyaline or subhyaline, and born from the phialide-like basal mother cell. Conidiogenous cells were short filiform lateral and germinated solitary aleuriospores that were unicellular, dark, oval, or broad concave lenticular or globose in shape, at one side of which was a conspicuous budding slit.

Type species: *Arthrinium caricicola* Kunze.

Arthrinium sphaerosperma (Per.:Fr.) Ellis (= *Papularia sphaerosperma* (Pers.: Fr.) vor Hohnel, new recorded-species of China (Ellis et al. 1951; Ellis 1965) in Fig 1-8

Characteristics: Colonies on PDA, 25 °C, were initially white, turning dark, and substratum was black. At the terminal of hypha formed many phialide-like mother-cells, out of which develop the narrow, thread-like conidaophores, smooth, with 5-60 µm in length and 0.5-1 µm in diameter, with 0-3 septa. Basal cells were usually ovoid or conico-truncate, hyaline when they were young, later became pale brown, smooth, about 4-8 µm in height and 3-4 µm in diameter. The conidia were aleospores, lenticular, smooth, brown with a bright budding rim on one side, (10-14)×(6-7) µm in size, germinates a germ tube, and (30-70)×(1-1.5) µm long.

Specimen of study: The bark HSSPD 1005 of *Taxus*

cuspidata Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(9) *Ambrosiella* Brader: von Avx & Henneber 1965, Mycopathologia 25: 312-305; Batra, 1967 Mycologia., 59:976-1017, new recorded-genus of China.

Characteristics: Colonies were woolly and compact and the color for white, yellow, to yellowish brown, gray, brown or rusty, etc. Thread-like mycelium initially was hyaline, but becoming grayish brown, and conidiophores were branched or unbranched, monilioid, arising from vegetative mycelium or arising by the transformation of germ tube from aleuriospores. The conidiophore gave rise to acropetal chain forming by thick-walled hyaline sprout cells or gave rise to a thick-walled, solitary, terminal globose to subglobose, and hyaline conidium. Conidium was solitary, terminal blastospore, but its teleomorphon was unknown.

Type species: *Ambrosiella xylebori* Brader ex von Arx & Hennebert.

Ambrosiella brunnea (Verrall) Batra, new recorded-species of China (Batra 1967) Fig. 1-I.

Characteristics: Colony, yeast-like raised in the center, is woolly, cerebriform, white to cream and substratum was golden yellow, subsequently becoming cassia to Raw Umber or fuscous. Mycelium can bear pseudomycelium consisting of branched or unbranched chains of globose or subglobose conidium-like sprout cells, with 7-10 µm in diameter. Pseudomycelia or their lateral branches often broke into smaller unites or gave rise to new sprout cells by fission or budding. Conidiophores were short, born from lightly brown vegetative mycelium, filiform, and measuring (10-42)×(3-4.5) µm, the terminal cell characteristically tapering toward the apex and measuring (16-24)×(3-3.5) µm. Conidiophores in tunnels formed golden yellow or fuscous sporeidia. Conidia, on the terminal of conidiophores, were solitary blastospore, globose to subglobose or broadly ellipsoid. Conidiophore gave rise to fission cells or germinated into monilioid chains of sprout cells. Conidia were hyaline, smooth-walled, with (7-17)×(8-12) µm in size and become brown, thick-walled, terminal ellipsoid conidia in older cultures.

Specimen of study: The bark HSSPD 1008 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

(10) *Cytosporina notha* Diedicke, H., Kryptogamenflora der Mark Brandenburg. IX, P.545, p.432, f. 36. Diedicke, H., 1912, Kryptogamenflora der Mark Brandenburg. IX, p. 545, p. 432, f. 36; Syn.: *Rhadospora notha* Saccardo. Syll. 3: 583, new recorded-species of China (Saccardo, 1884; Diedicke, 1912; Grave, 1935; Sutton, 1980) in Fig. 1-J.

Characteristics: Colonies on PDA, 25 °C, were round,

Cotton-like, radiat extension, and black stromas. Substratum was black near the fruiting body. Sporodochium grew from colony, oblate, 350-600 μm long, numerous cavities, with ostiole on the terminal of every cavity. The cavity-platform was unobvious pseudoparenchyma, slightly white inside. Conidium was line-like, curved, gradually tight on the terminal, slightly sharp on both sides, hyaline, and $(23-29) \times (1.2-1.4)$ μm in size. Conidiophore was cannon

and $(10-13) \times (1-1.2)$ μm in size.

Specimen of study: The bark HSSPD 1006 of *Taxus cuspidata* Sieb. et Zucc. was preserved in Bioengineering and Biotechnology Pharmaceutical Teaching-research department of Shenyang Pharmaceutical University.

Sources: The strains were from the Changbai Mountain Natural Reserve in Jilin Province.

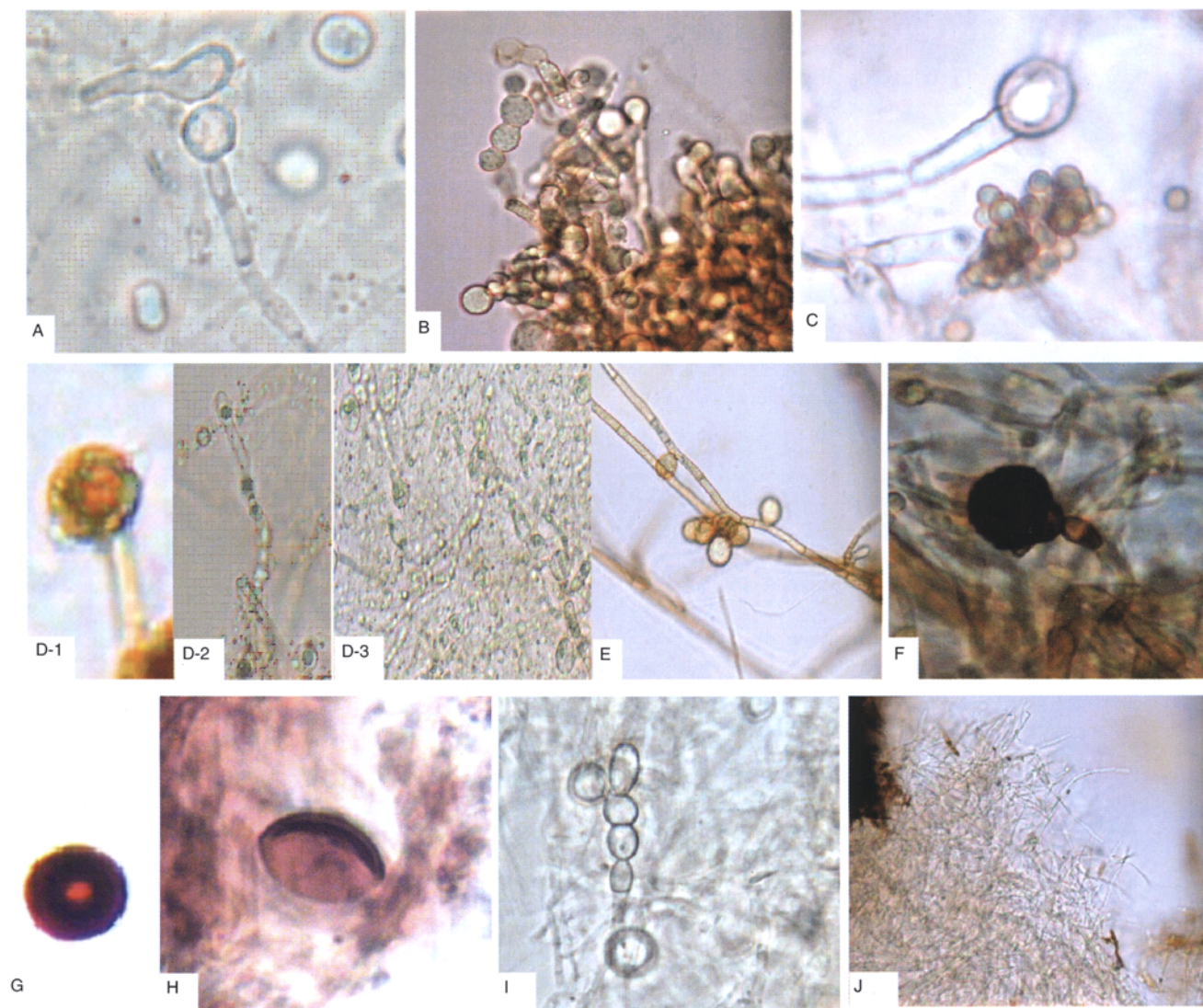


Fig. 1 New recorded-genus-species of China

- A. *Staphylotrichum coccosporum* Meyer et Nicot: conidiophores and conidia $\times 800$;
 B. *Botryodema lateritium* Papendoorf & Upadhyay: mycelium, branched conidiophores and conidia $\times 156$;
 C. *Oedocephalum glomerulosum* (Rulliard) Sacc.: conidiophores, heads of conidia $\times 760$;
 D. *Trichosporonoides oedocephalis* Haskins & Spencer: D-1 conidiophores and heads of conidia $\times 1000$, D-2 conidia and budding conidia $\times 500$, D-3 mycelium producing blastospores and arthrospores $\times 370$;
 E. *Beniowskia sphaeroidea* (Kal Chbrenner et Caeke) Mason: pseudomycelium, conidiophores and conidia $\times 160$;
 F. *Thermomyces verrucosus* Pugh, Blakeman & Morgan-jones: mycelium, conidiophores and conidia $\times 610$;
 G. *Gilmaniella humicola* Brion: mature conidia showing germ pore $\times 1000$;
 H. *Arthrium sphaerosperma* (Pers.:Fr.) Ellis: conidia and conspicuous budding rim on one side $\times 880$;
 I. *Ambrosiella brunnea* (Verrall) Batra: conidia geminate into monoid chains of sprout cells $\times 480$;
 J. *Cytosporium notha* Diedicke: cavity-platform, conidiophores and conidia $\times 160$.

The results of the fermentation metabolites of the strains

TLC results of the fermentation metabolites of the strains are as follows:

The strains whose fermentation metabolites reacted positively under UV 253.7 nm are in order of *Staphylotrichum coccosporum* Meyer et Nicot 1012, *Gilmaniella humicola* Brron 1022, *Ambrosiella brunnea* (Verrall) Batra 1008, *Trichosporonoides oedocephalis* Haskins & Spencer 1023, *Botryodema lateritium* Papendoorf & Upadhyay 1002, *Cytosporina notha* Died. 1006, *Thermomyces verrucosus* Pugh, Blakeman & Morgan-jones 1025, *Arthrimum sphaerosperma* (Per.:Fr.) Ellis1005, *Oedocephalum glomerulosum* (Rulliard) Sacc. 1016.

The fermentation metabolites of the strains could not react positively with taxoids developer - the vanillic aldehyde and dilute bismuth potassium iodid

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